

THE PHYSIOLOGY OF THE OVARIES*

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IT IS WELL recognized that the ovaries have a double function, the formation and periodic liberation of sex cells and the secretion of the female sex hormones, the estrogens and progesterone, which play an important role in the maintenance and cyclical changes of the accessory reproductive organs. It is also clear that the ovaries are not self-maintaining structures. Certain hormones of the anterior pituitary are essential to their maintenance and in turn the secretions of the ovaries influence the secretion of the gonadotropic hormones and probably other hormones of the hypophysis.

The inter-relations of these various structures have been intensively studied in the clinic and experimental laboratory for more than a decade. Although they appeared to be simple at first and in their basic outlines are simple, yet they have many complex features which even at the present time are but imperfectly known. Evidence for this last statement can be gained by even casual examination of results obtained in any sterility clinic, or of experimental work directed towards the reinstitution of fertility when sterility has been induced by the experimental ablation of the hypophysis. We also have, perhaps, been too inclined to believe that the ovarian hormones act solely on the accessory reproductive organs, whereas in fact these secretions are powerful agents which certainly influence many other body mechanisms, perhaps all of them.

The developmental and later cyclical changes of the structural components of the ovary need, as an introduction, to be only hastily reviewed. At birth an immense number of ova are present. Figures differ somewhat but show that there are between 100,000 and 400,000 in the human ovary at birth. Between birth and puberty many of these disappear but there still remains a large number at the onset of sexual maturity (Schroeder¹). In addition to this initial number, most recent investiga-

* Read November 2, 1939 at The New York Academy of Medicine in the Twelfth Graduate Fortnight. From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

tions indicate that there is a formation of new ova from the germinal epithelium throughout the reproductive period (Allen,² Evans and Swezy³ and others), although this is not universally accepted as being true (Kingsbury⁴). In the human, all ova are destined to mature or to undergo degeneration and destruction by or shortly after the end of the reproductive period, for within a short time after the menopause none can be found nor can follicular development be stimulated by the injection of gonadotropic hormone. It is evident that but few of the ova and their enclosing follicles have undergone complete development, the number of eggs that normally mature during the entire reproductive period of a woman not exceeding some 400. Thus most of the original thousands of primordial follicles degenerate at some stage in their life cycle, not infrequently after they have undergone considerable growth or indeed after they have reached nearly mature size. In most mammals the theca interna of those follicles which undergo atresia form cords or masses of epithelioid-like cells, the so-called interstitial tissue of the ovary. In rabbits in which this type of tissue reaches the greatest development, the ovaries are laden with these cells and they were designated as the puberty gland by Ancel and Bouin. That these cells can excrete estrogenic hormone in lower forms is evident from work on hypophysectomized rats. In old-world monkeys and humans, however, interstitial tissue is very scanty or absent and it is difficult to attach any secretory role to it. The hyalinized bodies which form from atresia of follicles in the human can hardly be suspected of having a secretory function.

The changes and differentiation of various parts of those follicles which are destined to undergo maturation and ovulation are well known, and will not be reviewed. It will suffice to refer only to the fact that during this growth two structures are differentiated which, in addition to the ovum, could, from their structure, be expected to form secretions. These are the epithelial lining of the follicle, the granulosa, and the inner layer of the enveloping connective tissue capsule, the theca interna. The cells composing the latter enlarge and assume epithelioid characteristics during the growth of the follicle. It has been thought that the follicular fluid which, as first shown by Allen and Doisy et al⁵ in 1924, contains estrogenic hormone, was probably formed by the granulosa. Corner,⁶ however, has recently reviewed the evidence in regard to which cells form estrogen and has come to the conclusion that the connective tissue element, the theca interna, and not the granulosa, is the site of

ovarian secretion of estrogenic hormone.

Following rupture of the follicle and extrusion of the ovum at ovulation profound changes take place in the follicle, resulting in the formation of a new structure, the corpus luteum. This newly formed structure secretes a second sex hormone, progesterone. Although a subject of debate at first, it now seems certain that corpora lutea also secrete estrogen.

In addition to the secretion of estrogen and progesterone, there is secreted by the human female a large amount of androgen, as shown by the urinary assays from the laboratories of Koch,⁷ of Laqueur⁸ and of Callow.⁹ That the ovaries may be the site of secretion of a part of this hormone appears probable. The experimental work of Hill¹⁰ in mice and of Deanesly¹¹ in rats has shown that ovarian grafts made into the ears of castrated males maintained the male accessory reproductive organs if the animals were placed in a cool environmental temperature. Due to their exposed position, the temperature of the transplanted ovaries was more influenced than they would be in their normal location. When the animals were kept in a warm room the accessory organs atrophied and assumed the condition characteristic of the castrated male, indicating that no androgen was secreted by the ovarian grafts. However, the evidence indicates that in women the most important site of formation of androgen is not the ovaries, but is probably the adrenal cortex. Hamblen et al,¹² using the colorimetric method of Oesting, found normal androgenic titers in five oöphorectomized women and Hirschmann¹³ chemically isolated dehydroandrosterone and androsterone from nine ovariectomized women in only slightly lower than the yields reported by Callow and Callow.¹⁴

Although estrogen is found in the blood and urine in ovariectomized women, as shown by Frank et al,¹⁵ Fluhmann¹⁶ and others, and estrone and progesterone normally occur in small amounts in the adrenals, it seems safe to assert that the effective estrogen and progesterone in non-pregnant women are formed in the ovaries, as evidenced by the profound involution of the accessory reproductive organs after ovariectomy. Where the estrogen which is found in the blood and urine after ovariectomy comes from is an unsolved problem, but the adrenals are a likely source.

Before taking up the physiological actions of the female sex hormones—the estrogens and progesterone—I will discuss in as simple a way as

possible the number of natural estrogens which occur, their structural formulae and the transformations which they undergo before being excreted in the body, or in other words, their metabolism. It is known that but little of an injected estrogen can be recovered from the urine and feces. Furthermore, much that can be recovered is in an inactive form. It has been conjugated and in order to render it again biologically active it must be hydrolyzed. The site in the body where this inactivation or detoxification takes place will also be discussed. I do not find in the literature a simplified description of the chemistry of the estrogens and it seems that an attempt to give such a description might be of value to those who are interested in the sex hormones but who, like the speaker, are not chemists.*

A very large number—running into the hundreds—of estrogenic substances are now known. Their structural formulae have been determined and they have been synthesized in the laboratory. They can be separated into those which occur in nature, or the natural estrogens, and those which do not occur naturally, but which are produced only in the chemical laboratory. Most of the latter are of low potency, although some give a reaction with small amounts of material, as, for example, stilboestrol. Only seven estrogens are known to occur in the tissues and fluids of animals. Of these seven, five are not found in the urine of women but occur in the urine of pregnant mares. Three only (estrone, estriol and estradiol) are found in the urine of women. The third one named—estradiol—has only recently been isolated from the urine of women and occurs in very small amounts. It has also been isolated from the urine of pregnant mares and, more important from the point of view of the metabolism of the estrogens, from the liquor folliculi of sow ovaries, Doisy and his group¹⁷ in 1935 having isolated 11 mg. using more than four tons of sow ovaries to secure this small amount of chemically pure hormone. Since this substance is the one chiefly found in the ovaries, it is thought to be the parent hormone of estrone and estriol, the two other hormones isolated in humans. The naturally occurring estrogens all have the same carbon skeleton, the cyclopentenophenanthrene nucleus (Figure 1). This same skeleton is found in other biologically active substances, bile acids, toad-poisons, androgens, adrenal cortex hormone, progesterone, and the sterols. The differences between these substances

* Invaluable assistance was received from Dr. Louis Levin in the preparation of the discussion on the chemistry of the estrogens and progesterone.

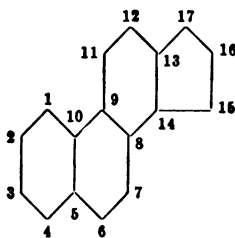


Fig. 1—The carbon skeleton (cyclopentanophenanthrene nucleus) of the naturally occurring estrogens and other steroid hormones, showing the numbering of the carbon atoms.

are due to different side chains and groupings attached to a common skeleton, and to certain space relationships. However, the difficulty of synthesizing the estrogens is much greater than it is with the androgens, progesterone and desoxycorticosterone. The latter are prepared commercially from the natural sterols such as cholesterol. The so-called pseudosynthesis of estrone from ergosterol has been reported but because of the cost of ergosterol and the small yield, it is not a practical method of obtaining estrone. The total synthesis of equilenine, an estrogen found in the urine of pregnant mares, has recently been reported by Bachmann, Cole and Wilds,¹⁸ and since equilenine can be reduced to estradiol the commercial synthesis of this substance appears to be a possibility. All the natural estrogens now in use come from urine, largely from the urine of pregnant mares. This urine yields largely estrone and this by reduction is changed to the more potent compound, estradiol.* Other substances may be substituted in one of the side chains, to give a more prolonged action, as for instance, estradiol benzoate (the commercial product called Progynon B), or estradiol dipropionate (Figure 2).

Chemically the three estrogens differ from each other only in the groupings on carbon 16 and 17 (Figure 2). Estradiol, the most active and probably the true follicular hormone, has an alcohol group on carbon 17. In estrone this alcohol group is replaced by a carbonyl (ketone) group. If the elements of water are added to estrone at the carbonyl group, estriol results. It is evident, then, that these three compounds are very closely related. In fact, some of the interconversions

* Estradiol occurs in two forms, α -estradiol and β -estradiol, the differences being due to the space relationships of the OH group on carbon 17. The α form predominates in the isomers obtained from the reduction of estrone to estradiol. This is fortunate for those who desire to make potent commercial preparations because the α form is much more potent (some 30 times) than the β form. Most commercial products of estradiol contain only the α form, the β form having been eliminated.

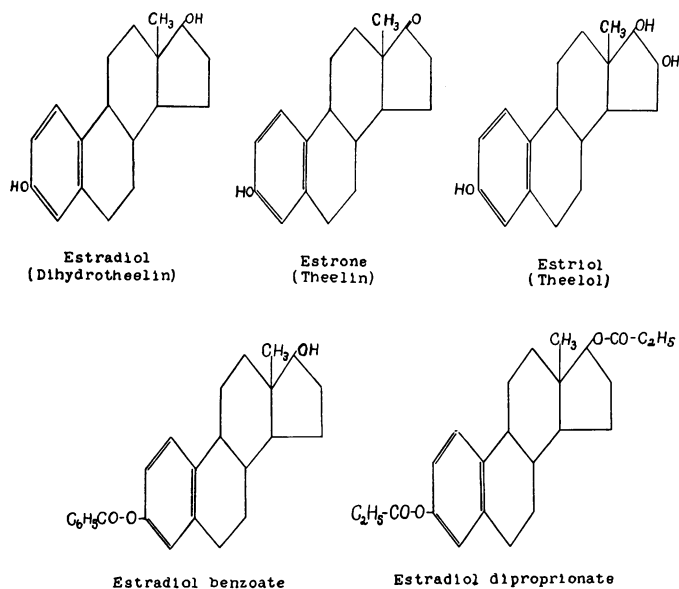
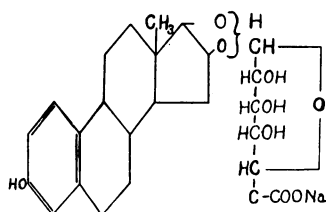


Figure 2

have been effected *in vitro* and demonstrated *in vivo*. Estradiol (dihydrotheelin) is the only one which has been actually isolated from ovarian tissue, as I stated earlier, and the activity of the follicular fluid is in a large measure accounted for by the estradiol content. The remaining fraction is ketonic in nature and is probably estrone. Estrone and estriol have been isolated from the urine of normal and pregnant women and from certain tissues of the body. These findings, in themselves, indicate that estrone and estriol may be the metabolites of estradiol. Further evidence supporting this view was furnished by Westerfeld and Doisy¹⁹ and by Pincus and Zahl.²⁰ They demonstrated the *in vivo* conversion of the hormones after injections into rabbits and monkeys.

The site of conversion or metabolism of the estrogen in the body is still uncertain. Pincus and Zahl claim that the conversion of estrone to estriol in the rabbit requires a functional uterus, for they could not demonstrate this conversion in hysterectomized or long-time castrated animals. Westerfeld and Doisy,¹⁹ however, found that the presence or absence of the uterus had no effect on the conversion of estradiol to estrone and vice versa. Whether or not these two sets of experiments deal with two different mechanisms is not yet known.



Estriol glucuronidate

Figure 3

The urinary estrogens are largely excreted in a combined form, as is indicated by the discovery that yields of estrogen from urine are markedly increased by preliminary hydrolysis. Marrian and co-workers²¹ have isolated the combined estriol from human pregnancy urine and found it to consist of estriol glucuronidate, the linkage involving the terminal aldehyde group of the glucuronic acid and the hydroxyl group of carbon 16 or 17 of the estriol. The complete explanation for this conjugation is not yet known. However, it is well known that glucuronic acid is used by the organism in detoxicating many substances. Also, the conjugation with glucuronic acid increases the water solubility of estriol. A third fact, demonstrated by Marrian²² is that the glucuronidate is relatively inactive as compared with the free estriol. Thus it may be that the conjugation is made by the organism in order to detoxicate the estriol, to increase its solubility in the urine or to inactivate the estrogenic potency or for all three reasons. That the inactivation may be of particular importance during pregnancy is suggested by Marrian's finding that shortly before the onset of labor the proportion of free estrogen rises very markedly. Marrian deduced from this that possibly the increase in free estrogen is for the purpose of sensitizing the uterus to the oxytocic principle of the pituitary and so to facilitate the onset of labor. During the first eight and one-half months of pregnancy there is no need for sensitization of the uterus to oxytocin and so the estriol is converted to the relatively inactive glucuronidate.

Estrone is also conjugated before excretion. Preliminary work by Schacter and Marrian²³ suggests that in pregnant mares' urine, estrone is present in combination with sulphuric acid. Whether or not this is also true for the estrone of human pregnancy urine is not yet known.

The nature of the estrogen conjugates of normal human urine is likewise unknown.

The site in the body where the estrogens are conjugated and so rendered inactive appears to be the liver. Zondek,²⁴ a number of years ago, showed that estrogen was inactivated when incubated *in vitro* with liver. Israel and his colleagues²⁵ found inactivation to occur in a lung-heart-liver perfusion system, but not in a lung-heart preparation. Golden and E. L. Sevringhaus²⁶ noted that in animals in which the ovaries were transplanted into the mesentery no evidence of heat appeared, and Biskind and Mark²⁷ showed that pellets of estrone implanted in the spleen when the circulation was intact gave no response, whereas if the splenic artery and vein were ligated the pellets were effective, presumably because of absorption by the non-portal part of the circulation. Although a role of the altered spleen cannot be overlooked in the inactivation of the estrone in these last experiments, nevertheless the evidence from these various experimental approaches seems definitely to show that the conjugation and consequent inactivation of the estrogens takes place in the liver. If an estrogen is always destroyed in its passage through the circulation of the liver, however, it is puzzling to understand how this substance, when given by mouth, can be effective. Estriol, it will be recalled, is quite effective by mouth in experimental animals, at least. Another puzzling finding is that of Dingemans et al.,²⁸ who have recently reported that considerable amounts of combined estrogen are found in ovaries. It seems quite unlikely that this could have been conjugated in the liver and then stored in the ovaries, although this is a possibility. If such is not the case the alternative remains of it having been actually secreted in the ovary in a combined inactive form.

The inactivation of estrogen by an organ such as the liver may partially explain the pronounced response from percutaneous or per-epithelial administration, even though the rate of absorption is low, for in this method of administration all the estrogen would act on the underlying structures before passing into the circulation and there being inactivated in its passage through the liver circulation.

The active substance peculiar to the corpus luteum, named progesterone, was isolated in 1934, simultaneously by four different laboratories. Its structure has been determined, showing it to belong to the same family of compounds as the estrogens. It is to be noted that progesterone has quite different active groupings from the estrogens (Figure

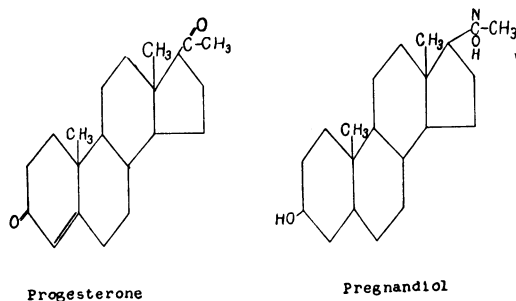


Figure 4

4). Instead of a phenolic hydroxyl on carbon 3, progesterone has a ketone group with a single unsaturated linkage in this position. On carbon 17, instead of a hydroxyl or ketone group, progesterone has an acetyl group. In addition, progesterone has the usual sterol methyl group on carbon 10. This, it will be recalled, is absent in the estrogens.

It is converted by the endometrium, according to Venning and Browne,²⁹ into an inactive compound, pregnandiol. These investigators report that during pregnancy and during the last half of the menstrual cycle, when luteal activity is high, pregnandiol is excreted in the urine. Similarly they find that injections of progesterone into ovariectomized women, previously sensitized by estrogen, result in pregnandiol excretion. However, no pregnandiol could be demonstrated after administration of progesterone into hysterectomized women. This led Venning and Browne²⁹ to postulate that the reduction to pregnandiol takes place in the endometrium. However, recent work by Buxton and Westphal³⁰ indicates that other mechanisms for the conversion are possible. They find that normal and pathological men, treated with progesterone, excrete almost theoretical amounts of pregnandiol. In these cases, certainly, the conversion could not take place in the uterine endometrium.

Venning and Browne have advocated the use of urinary pregnandiol determination as a measure of corpus luteum activity. That the determination has a great value is undeniable. However, it should be remembered that the pregnandiol is but the end product of the chemical reduction of progesterone. A large number of intermediate products is possible—in fact, many of them have been isolated from urine. It therefore appears that negative findings as to pregnandiol excretion need not necessarily mean lack of luteal activity. It is quite possible that pro-

gesterone may be secreted at a normal rate by the corpus luteum but that its conversion to pregnandiol is incomplete. Thus, one would find little of this compound in the urine, although a careful search might show the presence of other degradation products of progesterone. It is evident that in order to determine the presence or absence of active corpora lutea, pregnandiol determinations should be supplemented by uterine biopsies.

THE ACTION OF ESTROGEN

The estrogens are powerful pharmacological agents. A response of the female accessory reproductive organs was the first one noted and is the most prominent effect and so it is quite logical that it has been the one on which attention has been primarily focused, and effects on other structures neglected. The few studies which have been made on other responses make it unsafe to assume, however, that only the reproductive system, including the mammary gland, is affected by these hormones. These remarks could be justified even though no relationship to the genesis of cancer had been established. The reaction induced by the various estrogens is similar, differing only in intensity and duration, and so can here be treated as due to a single substance.

The action of estrogen on the uterus is well known and need be only briefly discussed. The uterus after ovariectomy undergoes profound involution; the glands become short and few in number and the epithelium of a low non-secretory type. If estrogen is administered all parts of the organ are stimulated. The myometrium becomes thicker and more vascular. The endometrium particularly responds, becoming thickened and showing a growth in the number and length of its glands, and some glycogen deposition takes place in the basal zone of the gland cells. The epithelium is increased in height. The vascular response is immediate, pronounced and striking, as shown by direct observation of the uterus in rabbits through an abdominal window and of endometrial transplants in the anterior chamber of the eye (Markee,³¹ Pompen³²). There is an early increase in the acetylcholine content, as shown by the fact that atropine will inhibit the dilatation of the vascular bed and by tests on eserized frog muscle (Pompen,³² Reynolds³³). The endometrium imbibes fluid from the dilated and slowed circulation. These vascular changes precede the later enhanced growth and metabolic changes. The resultant effect is then a proliferation of epithelial cells and a thick-

ened and a highly vascular and edematous endometrium. For the development of the type of endometrium in which implantation of the fertilized egg can take place, the second female sex hormone, progesterone, is necessary. Although its first action, as shown by experimental work, is to increase mitoses in the glands and epithelial lining of the uterus (Lloyd³⁴), its later and specific effect is to bring about a secretory condition in this epithelium. Glycogen, which lies in the basal portion of the gland cells in the estrogen phase, is transferred to the apical zone and together with mucus is secreted into the lumina of the glands. The lumina become not only filled with this secretion but become enlarged and irregular in shape.

Although the induction of this secretory or progravid state of the endometrium is a specific action of the corpus luteum hormone, its action is enhanced by estrogen, that is, the amount of progesterone necessary to produce a progravid endometrium is decreased by injecting estrogen (Hisaw et al,³⁵). Engle and Smith³⁶). * Not only is the progesterone response enhanced by estrogen, but, as has been experimentally shown, the progravid condition can be maintained for a longer period if estrogen is concurrently administered. This work, which has shown that the progesterone response is enhanced by estrogen, thus correlates with what I have described earlier, namely, that the corpus luteum normally secretes both progesterone and estrogen.

* In earlier work (Hisaw and Leonard,³⁷ Clauberg,³⁸ Robson³⁹) on the combined action of estrogen and progesterone on the endometrium of rabbits, it was found that the progestational effect of progesterone was nullified by the concurrent injection of relatively small amounts of estrogen. Estrogen is pronouncedly antagonistic to progesterone in rabbits and the progestational action of the latter is nullified if about 1/75 to 1/100 of its weight of estrone is administered, or in international units, a ratio of about 1 to 100. However, even in rabbits the addition of some estrone to the progesterone after a progravid condition had been developed by progesterone alone, prolongs the progravid condition (W. M. Allen⁴⁰). Normally the progravid condition induced by progesterone administration in spayed rabbits disappears by the tenth day. In order to secure a progesterone response it is necessary, of course, to sensitize the uterus by a preceding series of estrogen injections.

In monkeys the estrogen-progesterone ratio which will prevent the progestational reaction is much higher than in rabbits. A daily dosage of estrogen as high as 500 R.U. or approximately 2500 to 5000 I.U. does not inhibit the action of .5 I.U. of progesterone, a ratio in I.U. of 5000-10,000 to 1 (Allen, Hisaw and Gardner⁴¹). These findings in monkeys appear to correlate much better with those in the human than does the work on rabbits, although no precise experiments have been reported in the human. Not only is it well known that corpora lutea in the human contain much estrogen, but in addition assays of blood and urine show a much larger amount of estrogen than can be found in lower forms, including monkeys. It seems safe to assume that the action of the progesterone from the human ovary is increased and not inhibited in the human by the circulating estrogen.

I would like to point out, however, that the response of the endometrium does not depend solely on the action of estrogen and progesterone, but is influenced by altered body states. In hypophysectomized monkeys we have found that the response to these hormones is less than in spayed animals and that the latent period between withdrawal of both estrogen and of progesterone and menstruation is very greatly increased. The cachexia and other disturbances induced by hypophysectomy evidently reduce the quantitative responses to these hormones, although qualitatively their actions are not changed.

Curious as it may seem, work has established that estrogen assists in the structural and presumably the functional maintenance of the corpus luteum itself. I refer to the work of Westman⁴² and of Robson⁴³ in rabbits in which it was shown that the rapid regression of the corpora which characteristically occurs after hypophysectomy can be prevented by the administration of estrogen. In such an experiment it is obvious that this maintenance cannot be indirectly due to a stimulation of the hypophysis. Although an indirect effect through other endocrine glands has not been disproven, nevertheless this work indicates that estrogen, an ovarian secretion, assists in the maintenance of an ovarian structure, the corpus luteum. This could justifiably be characterized as a gonadotropic effect of estrogen. This corpus luteum maintenance effect of estrogen, however, is not permanent and regression of the corpora ultimately occurs, even though the injection of estrogen is continued. Regression which would normally occur within five days is postponed only until the thirteenth to the sixteenth day, which is the period that corpora lutea remain functional in pseudopregnancy in rabbits. We have to admit that the reactions of the reproductive organs of rabbits are peculiar in some respects and that this gonadotropic effect of estrogen may not hold in monkeys and the human. The presumption is, however, that the same response will be found in these higher forms.

It seems desirable to mention some of the hormonal conditions obtaining in pregnancy, for not only is the maintenance of the state of pregnancy dependent on the continued secretion of the ovaries in most forms, but the newly formed organ, the placenta, is a secretory organ and supplies hormones normally secreted primarily by the ovary. In most laboratory animals abortion soon takes place, when the ovaries are removed. Such is not the case in the human, in monkeys, in guinea pigs and in mares. Except in monkeys, the literature is sufficiently extensive

and definite to establish that the ovaries are not essential for the continuation of pregnancy in these species.

A number of years ago Robert Frank pointed out that the placenta probably had a secretory function, and this has been found to be the case. It appears to be highly probable, and indeed established, that the gonadotropic hormone which appears in the urine in large amounts early in pregnancy in women is formed by the placenta. The designation of this hormone as chorionic gonadotropin is being widely adopted and this name was accepted at the Third International Conference on the Standardization of Hormones held in Geneva last year. Estrogen continues to be present after the removal of the ovaries during pregnancy, although further studies are desirable to determine if it is in normal amounts and how great a reduction invariably occurs after ovariectomy. The placenta contains a higher concentration of estrogen, mostly as estriol, than any other organ of the body.

Less work has been done on the placental source of progesterone than is the case with the estrogens. A method for the assay on the excreted degradation products of progesterone has only recently been devised, and though it has already proven of great value in supplying additional evidence on the period when progesterone is secreted in the normal cycle and during pregnancy, nevertheless but little work on the effect of ovariectomy on the secretion of progesterone in pregnant women has been done. Browne et al⁴⁴ and Jones and Weil⁴⁵ have reported pregnandiol excretion after excision of the corpus luteum during pregnancy. In the case of Jones and Weil there was a temporary drop followed by a return to nearly normal levels. Even though data do not conclusively show that the placenta secretes estrogen and progesterone, it appears highly probable that such is the case and that in the special condition of pregnancy the placenta supplements and indeed can take over in the human the secretion of the female sex hormones in the absence of the ovaries.

I shall not include a discussion of the effects of estrogen and progesterone on the two other main structures of the reproductive system, the vagina and mammary glands. Proliferation of the epithelial elements of both organs is caused by estrogen. In the vagina there is an increased deposition of glycogen and a consequent lowering of the pH. This increased acidity and accompanying epithelial proliferation and cornification is probably responsible for the benefit in gonorrheal vaginitis.

As stated earlier in the paper, the extra-reproductive effects of estrogen have not been investigated nearly as extensively as have the responses of the reproductive organs. Studies, it is true, have been made on such subjects as the influence of estrogen on ketosis, carbohydrate, fat and basal metabolism, serum calcium, and blood formation, but not only is more work required before significant or at least definite conclusions, in most cases, can be drawn, but also any indirect effect through the pituitary gland must be further tested, so that it would be premature to attempt to draw conclusions at this time.

Although blood pressure determinations after estrogen injections are negative and consequently an effect on the larger blood vessels and the heart is improbable, nevertheless significant and definite changes have been reported in the peripheral circulation. Work on the peripheral vessels was stimulated from two findings. First, injection of estrogen causes within an hour in rabbits a pronounced hyperemia. This is not due to a heightened metabolism of the tissue, for the hyperemia precedes an increase in oxygen consumption. Secondly, the hyperemia from estrogen injection is not limited to the uterus but is participated in by the sexual skin in monkeys and by the nasal mucosa. The hyperemia persists throughout the estrogen injection and leads at least in the uterus and the sexual skin of monkeys to water retention and a high osmotic pressure of the interstitial fluid (Aykroyd and Zuckerman⁴⁶) and sodium retention (Thorn and Harrop⁴⁷). In a form in which the sexual skin changes are pronounced, the water retention is sufficient to produce a significant increase in body weight (Krohn and Zuckerman⁴⁸). The weight increase is accompanied by a decrease in urine output. Although a change in the water content of the skin is more pronounced in forms having a distinct swelling of the sexual skin, nevertheless an effect on water retention from estrogen injections has also been shown to occur in rats, a species in which there is no external manifestation of changes in the skin (Zuckerman et al⁴⁹). This shift in water to the skin in rats amounts to almost 1. per cent of the body weight and is accompanied by slight decrease in the water content of muscle and certain of the organs. This water retention effect of estrogen is also induced by other sterol hormones, although certain differences, as in nitrogen retention, occur and Zuckerman⁵⁰ states that the excretion of water which has accumulated in the sex skin of monkeys from estrogen administration is not prevented by progesterone, testosterone or cortin. The effect of estrogen

on the peripheral circulation has been further explored by Reynolds and his co-workers in rabbits and the human. By direct observation on the transilluminated ear of rabbits Reynolds and Foster^{51, 52} observed that injection of estrogen caused a dilatation of the capillaries and small venules. The ear became pink. That there was usually not an increased blood flow was evidenced by a fall rather than a rise in the temperature of the ear. The effect was limited solely to the small peripheral vessels as there was no constant change in blood pressure. In measurement of the volume of the finger in men they (Reynolds and Foster⁵³) found an increase which averaged 4.6 per cent in twelve subjects, whereas in six there was no response. Although the increase was small, attention was called to the fact that the fleshy part of a finger comprises only about one-half the total volume of this digit, and an increase in size would be limited to the soft part and not to the hard part—the nail and bone. Although work on the peripheral vascular responses has not been extensive, yet sufficient work appears to have been done to establish that vascular effects are not limited to the sexual organs of the female.

The mechanism by which estrogen alters the peripheral vascular bed, so far as it has been elucidated, seems to be an interesting one. Pompen³² found that the heightened vascularity of the uterus induced by estrogen injections would be inhibited by atropine sulphate. This indicated that the response might be due to an increase in acetylcholine. Reynolds⁵¹ has carried out a series of assays of the acetylcholine content of the uterus. He finds that in ovariectomized rabbits no trace of this substance could be found in most cases, whereas within an hour after an injection of an estrogen (Amniotin, Squibb) a very appreciable amount was present. Whether, in the general peripheral vascular bed, acetylcholine is the agent responsible for the dilatation of the small vessels remains yet to be established. That such may be the case is indicated by the interesting findings reported by Soskin and Bernheimer⁵⁴ in atrophic rhinitis. These investigators postulated that the beneficial effects in atrophic rhinitis reported by Mortimer et al⁵⁵ and by Blaisdell,⁵⁶ from injections of estrogen, were due to hyperemia. They tested this hypothesis not by local application of acetylcholine, which might be dangerous, but by potentiating that which was normally present but which is inactivated by choline esterase. They inhibited the action of the latter with local applications of a physostigmin-like substance. In a moderate-sized series, fourteen patients, this treatment, they report, gave better

results than were secured with local applications of estrogen. If further work confirms this report, it will be an excellent example of an immediate practical application of findings which appeared to be of theoretical physiological interest only.

Among the extragonadal effects of estrogen the action on the anterior pituitary gland is outstanding. This action is so well known, however, that a brief discussion will suffice.

It was early noted that the daily subcutaneous injection of estrogenic preparations damaged the reproductive apparatus of male animals. At first this was attributed to a hormone antagonism but when it was later found that ovarian atrophy also resulted, it seemed likely that the injury was due to decreased hypophyseal activity. This view was strengthened by the discovery that the injection of gonadotropic hormone concurrently with estrogen prevented the ovarian atrophy in normal animals and that in hypophysectomized rats estrogen administration did not decrease the effect of gonadotropic hormones. The physiological evidence seemed to show clearly a depressant effect on the hypophysis. Evidence soon appeared, however, showing in certain but not in all forms that estrogen caused a formation of corpora lutea, a finding which would indicate an enhanced output of luteinizing hormone, according to the ideas current about the two hypophyseal gonadotropic hormones. There was thus suggested a reciprocal relationship between the ovaries and the hypophysis, which would very nicely explain the rhythmicity of the female sex cycle. With the growth of the follicles in the first half of the cycle there would be increasing secretion of estrogen which in turn would depress the output of follicle-stimulating hormone and increase the secretion of luteinizing hormone. As the correct balance between these two gonadotropic hormones was reached, rupture of the follicles would result and corpora lutea would form and be maintained by the luteinizing hormone. Such a concept, as shown by subsequent work, is far too simple. Luteinizing A.P. hormone, as prepared by present methods, does not maintain the corpora lutea, but rather in some forms, at least, it causes their disappearance. The lactogenic hormone, on the other hand, induces the functional and structural persistence of the corpora lutea. The ovarian-hypophyseal interrelationship in the cycle is apparently not as simple as was outlined. The marshalling of additional data frequently spoils these simple *schemata*.

However, both experimental and clinical work show that estrogen,

when given in considerable amounts, represses the liberation of at least one of the two or more gonadotropic hormones supposed to be secreted by the hypophysis. Even this finding, which seems clear from all physiological evidence, is difficult, however, to harmonize with the structural changes which occur in the hypophysis, for these changes definitely indicate an increased activity of the basophiles. However, it is certain that the administration of estrogen decreases the urinary output of pituitary gonadotropic hormone which normally rises at the menopause, a rise which has been given as the cause of the menopausal disturbances. This may well be too simple an explanation, for the influence of estrogen is not limited to the hypophysis and the reproductive system as pointed out earlier in this paper.

The repressing effect of estrogen on the hypophysis is not limited to the gonadotropic hormone or hormones. A number of experimental studies have shown a slowing or indeed a stoppage of the growth in young animals. Although there are differences of interpretation in regard to the changes in the epiphyseal cartilages from estrogen administration (Tausk and de Fremery,⁵⁷ Zondek,⁵⁸ Pfeiffer and Gardner,⁵⁹ Silberberg and Silberberg⁶⁰), it can be stated that, whatever may be the nature of these changes, it is certain an abrupt stoppage of growth can be induced in experimental animals (Deanesly⁶¹ and others). The injection of estrogen, however, could hardly be recommended as a method for limiting growth in the human, because the effects of this hormone are general, as I have emphasized earlier in this paper. In addition to the depressant effects on growth, we can add the more questionable one of depression of the thyroid and the induction of an adrenal hypertrophy.

In this paper I have limited myself to a discussion of the action of the ovarian hormones, for this subject is sufficiently complex to make me well-satisfied to have had the pituitary gonadotropic hormones and their action on the ovaries discussed earlier in the Fortnight series by J. B. Collip. I may, perhaps, have appeared to overemphasize the complexity of the action of estrogenic hormones, although I believe such is not the case. I have tried to point out that the estrogens—certainly much more so than progesterone—are powerful pharmacological agents. Even though we overlook the possibility of carcinogenic effects, they cannot be given with assurance that their action will be circumscribed or limited. The advance in our knowledge about the action of the female sex hormone has been rapid, for it is only in the last few years that these substances

have been made available in quantities sufficient to carry on adequately experimental and clinical investigation with them. Since these hormones are powerful pharmacological agents and have a widespread action on the body, it is important to keep in mind that they should not be promiscuously given and that it is important to know when they should not be used as well as to know when they may be of value.

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